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Involvement of orexin-1 receptors in the ventral tegmental area and the nucleus accumbens in antinociception induced by lateral hypothalamus stimulation in rats

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ABSTRACT

Previous studies have demonstrated that chemical stimulation of the lateral hypothalamus (LH) with carbachol has an important role in the induction of antinociception in tail-flick test as a model of acute pain. In this study, we tried to evaluate the involvement of orexin-1 receptors in the ventral tegmental area (VTA) and the nucleus accumbens (NAc) on antinociceptive responses induced by LH stimulation in rats. One hundred twenty adult male albino Wistar rats weighing 200–250 g were unilaterally implanted with two separate cannulae into the LH, and VTA or NAc. Antinociceptive effects for two doses of intra-LH carbachol (125 and 250 nmol/0.5 μl saline), as a cholinergic agonist, were evaluated in this study. In another set of experiments, animals received intra-VTA or -NAc infusions of SB334867 as a selective orexin-A receptor antagonist (0.3, 1, 3 and 10 nmol/rat), just 5 min before microinjection of an effective dose of carbachol into the LH. In the tail-flick test, antinociceptive responses of drugs were obtained by tail-flick analgesiometer and represented as maximal possible effects (%MPE) at 5, 15, 30, 45 and 60 min after their administrations. The results showed that unilateral intra-LH administration of carbachol (125 and 250 nmol/rat) induced antinociception in rats ($P < 0.01$). There were no significant differences between the antinociceptive effects of these two doses. In the second part of our study, intra-VTA and intra-accumbal administrations of different doses of SB334867, 5 min before microinjection of carbachol, could dose-dependently prevent the development of LH stimulation-induced antinociception in rats. However, this effect was less in the NAc. It is supposed that the orexinergic projections from the LH to the VTA and NAc are direct/indirectly involved in the antinociception induced by LH chemical stimulation, and orexin-1 receptors in the ventral tegmental area have a more substantial role in this phenomenon.

1. Introduction

Our previous study has shown that the stimulation or inactivation of lateral hypothalamus (LH) produces antinociception (Safari et al., 2009). Several lines of evidence demonstrated that electrical and chemical stimulation of the LH can lead to levels of antinociception (Behbehani et al., 1988; Holden and Naleway, 2001). This area also has an important role in the modulation of nociceptive transmission at the level of the spinal cord (Aimone and Gebhart, 1987). One of the most important neurotransmitters in the LH is orexin. In fact, these neuropeptides are exclusively made in LH neurons that are projected throughout the central nervous system (CNS) (Lu et al., 2000; Marcus et al., 2001; Trivedi et al., 1998). Orexinergic projections from the LH (Mondal et al., 1999; Nambu et al., 1999) are innervated the different areas in the CNS such as the ventral tegmental area (VTA), nucleus accumbens (NAc), hippocampus and prefrontal cortex (Fallon and Moore, 1978; Lindvall and Bjorklund, 1974).

The orexins (also known as hypocretin) are neuropeptides transmitters that comprise of two distinct peptides called orexin-A and orexin-B. These neuropeptides result from prepro-orexin molecule during the proteolytic process (de lecea et al., 1998; Sakurai et al., 1998) which then activate two groups of G-protein coupled receptors: the orexin 1 (Ox1r) and 2 (Ox2r) receptors (Sakurai et al., 1998). Ox1r is selective for orexin A whereas Ox2r is willing for both orexins A and B (Sakurai et al., 1998). These neuropeptides are produced by orexin-expressing neurons which are frequently located in the posterior hypothalamus and extend to the dorsomedial and lateral hypothalamus (Peyron et al., 1998). The orexinergic system is most strongly associated with feeding, arousal, sleep, reward, addiction, stress, and pain processing (Azhdari Zarmehri et al., 2011; Harris et al., 2005; Petrovich and Gallagher, 2003; Safari et al., 2009; Sakurai et al., 1998; Sharf et al., 2010; Taslimi et al., 2011). The LH is associated with many nuclei which are effective in antinociception (Azhdari Zarmehri et al., 2011; Safari et al., 2009). Furthermore, it has been shown that the hypothalamic

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orexin system acts through the mesocorticolimbic pathway via many brain regions, but most studies have focused on the VTA (Sharf et al., 2010).

Based on previous reports in the VTA, the orexinergic projections infrequently synapse onto dopamine (DA) and GABA neurons. Nonetheless, these synapses have numerous dense orexin-containing core vesicles suggesting non-synaptic effects (Balcita-pedicino and Sesack, 2007). Both kinds of orexin receptors (Ox1r and Ox2r) have been found at high density in the VTA (Lu et al., 2000; Narita et al., 2006). In the VTA, orexins stimulate both DA and non-DA cells via a direct postsynaptic effect. Orexin receptor activation in the VTA could increase dopamine release via projecting neurons (Korotkova et al., 2003; Nakamura et al., 2000) in the NAc which is an important neuronal substrate of pain modulation (Breese et al., 1987).

The NAc is divided into the core and shell, and both of them directly or indirectly receive projections from the VTA (Deutch and Cameron, 1992). It seems that the functional interaction between NAc and VTA is implicated in pain processing, it is likely that the NAc is also involved in nociception (Magnusson and Martin, 2002). Additionally, both Ox1r and Ox2r have also been reported to be expressed in the NAc (Martin et al., 2002). There are several reasons to believe that the analgesia induced by substance P (SP), by the release of DA from terminals in the NAc (Kalivas, 1985) and intra-VTA infusion of SP increase DA metabolism in the NAc (Cador et al., 1989). Moreover, intra-VTA infusions of μ-opioid receptor agonists stimulate locomotor activity (Joyce et al., 1981: Kalivas et al., 1983; Latimer et al., 1987), and intra-NAc DA receptor antagonists or lesions made to the NAc, block this effect (Stinus et al., 1980; Kalivas et al., 1983). Therefore, considering the studies mentioned above, in the present study, we tried to examine the involvement of orexin-1 receptors within the VTA and NAc in antinociception induced by LH stimulation by the tail-flick test as an animal model of acute pain.

2. Materials & methods

2.1. Animals

One hundred twenty adult male albino Wistar rats (Pasteur Institute, Tehran, Iran) weighing 200–250 g were used in these experiments. The vivarium was maintained on a 12:12 h light/dark cycle at a room controlled temperature (23 ± 1 °C) with free access to chow and tap water. The animals were randomly allocated to different experimental groups. Each animal was used only once. Rats were habituated to their new environment and handled for 1 week before the experimental procedure started. All experiments were executed in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No. 80–23, revised 1996) and were approved by the Research and Ethics Committee of Shahid Beheshti University of Medical Sciences.

2.2. Stereotaxic surgery

Animals were unilaterally prepared with a guide cannula implantation at least 5–7 days before their use. The rats were anesthetized with intraperitoneal (i.p.) injection of Ketamine 10% (100 mg/kg) and Xylazine 2% (10 mg/kg) and cannulae were stereotaxically implanted in the LH, and the NAc or VTA (Stoelting, USA). The coordinates for these regions were determined by the rat brain atlas (Paxinos and Watson, 2007), AP = 4.8 mm caudal to bregma, Lat = ±0.9 mm lateral to midline, DV = 8.3 mm ventral from the skull surface for VTA (cannula 23-gauge, 11 mm, guide cannula was 1 mm above the appropriate injection place) and for the NAc (cannula 23-gauge, 11 mm) was AP = 1.7 ± 0.5 mm to bregma, Lat = ±1.6 mm lateral to midline, DV = 7.8 mm ventral from the skull surface and for the LH (cannula 23-gauge, 12 mm) was AP = 3 mm caudal to bregma, Lat = +1.6 mm and DV = 8.8 mm ventral from the skull surface. The guide cannulae were secured in place using two stainless steel screws anchored to the skull and dental acryl cement. After the cement was completely dried and hardened, two stainless steel stylets were used to occlude the guide cannulae during recovery period. Penicillin-G 200,000 IU/ml (0.2–0.3 ml/rat, single dose, intramuscular) was immediately administered after surgery. Animals were individually housed and allowed to recover for 5–7 days before experiments.

2.3. Drugs

In the present study, carbachol (Sigma–Aldrich, USA) as a cholinergic agonist was dissolved in physiological saline, and SB334867 (Tocris Bioscience, Bristol, UK), OX1 receptor antagonist, was dissolved in dimethyl sulfoxide (DMSO; Sigma–Aldrich, Germany). Control animals received either saline or DMSO as vehicles. All drugs were freshly prepared on the day of experiment.

2.4. Drug administration

Microinjections were performed by lowering a stainless steel injector cannula (30-gauge needle) with a length of 1 mm longer than the guide cannulae into the LH and VTA or NAc. The injector cannula was connected to a 1-μl Hamilton syringe by polyethylene tubing (PE-20), then drug solution or vehicle was unilaterally infused over 60 s and was left for 60 s extra time and followed by replacement of the obdurator. Different doses of carbachol were administered in a total volume of 0.5 μl/rat over a period of 60 s into the LH, slowly. SB334867 solutions (with DMSO as a vehicle) were administered in a total volume of 0.3 and 0.5 μl over a period of 60 s into the VTA and NAc, respectively. Injection needles were left in place for an additional 60 s to facilitate diffusion of the drugs, and then the stylets were reinserted into the guide cannulae. All drug solutions were freshly prepared on the test day, and all microinjections were unilaterally performed.

2.5. Tail-flick test

The nociceptive threshold was measured by the tail-flick apparatus (Harvard Apparatus, USA). Tail-flick test is an animal model of acute pain. The heat was applied in succession after the 3, 5 and 7 cm from the caudal tip of the tail. The value of each tail-flick latency (TFL) time was calculated on the average of three consecutive TFL tests in each time point. The reaction time between the onset of heat stimulus and the movement of tail was determined by an automatic sensor as TFL. The light source was set at an intensity that yields baseline TFL values in the range of 3–4 s (about 45% of maximal light intensity). If the animal failed to flick its tail within 10 s (cut-off point), the tail was removed from the coil to prevent damage to the skin (Haghparast et al., 2012; Ebrahimzadeh and Haghparast, 2011; Parvishan et al., 2011). TFLs (sec) are expressed either as raw data or percentage of maximal possible effect (%MPE) which was calculated from the following formula:

$$\%\mathrm{MPE} = \frac{\text{Post} - \text{drug latency (sec)} - \text{Baseline latency (sec)}}{\text{Cut} - \text{off value (sec)} - \text{Baseline latency (sec)}} \times 100$$

2.6. Experimental design

In this study, there were three control groups including intact, sham-operated and saline groups (n = 6 in each group) for determining the baseline TFLs, surgical manipulation and microinjection volume effects, respectively. To evaluate the involvement of OX1 receptors within the VTA and NAc in antinociceptive responses induced by LH stimulation, tail-flick test was performed as a model of acute pain. In all above control and experimental groups, TFLs

were recorded at 5, 15, 30, 45 and 60 min after drugs/vehicles administrations (Safari et al., 2009).

2.6.1. Dose–response effects of carbachol microinjected into the LH on tail-flick test

In these experiments, a dose–response relationship for carbachol as a LH chemical stimulation agent on tail-flick was established. Different doses of carbachol (125 and 250 nmol/0.5 μl saline; n = 6 in each group) microinjected into the LH, were tested (Holden and Naleway, 2001). Control animals received saline (n = 6).

2.6.2. Effects of intra-VTA administration of OX1 receptor antagonist, SB334867 on antinociception induced by intra-VTA administration of carbachol

To test the role of OX1 receptors within the VTA in the LH stimulation-induced antinociception, different doses of SB334867 (0.3, 1, 3 and 10 nmol/0.3 μl DMSO; n = 5–6 in each group) were unilaterally injected into the VTA, 5 min prior to effective dose of intra-LH carbachol administration, which was followed by a tail-flick test after 5 min. In the vehicles group (n = 6), DMSO was microinjected into the NAc and 2 min later animals received saline in the LH.

2.6.3. Effects of intra-NAc administration of OX1 receptor antagonist, SB334867 on antinociception induced by intra-VTA administration of carbachol

To test the role of intra-accumbal OX1 receptors in the LH stimulation-induced antinociception, different doses of SB334867 (0.3, 1, 3 and 10 nmol/0.5 μl DMSO; n = 6 in each group) were unilaterally injected into the NAc, 5 min prior to infusion of effective dose of intra-LH carbachol. In the vehicles group (n = 6), DMSO was microinjected into the VTA and 2 min later, animals received saline in the LH.

2.7. Histology

After performing the test, the animals were deeply anesthetized with Ketamine and Xylazine. Then, they were transcardially perfused with 0.9% saline and 10% formalin solution. The brains were removed, blocked and cut coronally in 50 μm sections through the cannulae placements. The neuroanatomical locations of cannulae tips were confirmed using Paxinos and Watson rat brain atlas (Paxinos and Watson, 2007). Only the animals with correct cannulae placements were included in the data analysis.

2.8. Statistics

The obtained results are expressed as mean ± SEM (standard error of mean). The mean TFLs in all groups were subjected to one-way and/or two-way ANOVA followed by protected Tukey's or Bonferroni's test for multiple comparisons, respectively. The mean maximal possible effect (%MPE) of drugs, as an analgesic index, was subjected to unpaired student t-test for comparison of two independent groups at time set intervals. In order to evaluate the nociceptive responses, area under the curve (AUC) was calculated as raw pain scores × time by linear trapezoidal method and a single value was used in statistical analyses. The calculated AUC and pain score values in all groups were subjected to one-way and/or two-way ANOVA followed by protected Tukey's or Bonferroni's test for multiple comparisons, respectively. *P*-value less than 0.05 was considered to be statistically significant.

3. Results

3.1. Dose–response effects of chemical stimulation of the lateral hypothalamus by carbachol on tail-flick test as an animal model of acute pain

In this set of experiments, we examined the dose–response effects of two doses of carbachol (125 and 250 nmol/rat) microinjected into the LH for the chemical stimulation of LH on TFLs in the rats. Two-way ANOVA for repeated measures over time followed by Bonferroni's test for %MPEs indicated a significant differences in antinociceptive responses to carbachol microinjected into the LH alone compared to those of respective saline-treated group [treatment effect: $F(2, 70) = 31.33$, $P < 0.0001$; time effect: $F(4, 70) = 0.5388$, $P = 0.7077$; interaction: $F(8, 70) = 0.4991$, $P = 0.8529$; Fig. 1A]. Moreover, as shown in Fig. 1B, one-way ANOVA followed by Newman–Keuls multiple comparison test showed that there are significant differences in mean AUC calculated values for %MPEs [$F(2, 16) = 22.4$, $P < 0.0001$] among the experimental (different doses of carbachol) and control (saline) groups. However, there was no significant difference in AUC calculated values for %MPEs as an index of antinociception between these two doses of carbachol. Therefore, we used the lower dose of carbachol, 125 nmol/rat, in the other experimental groups.

3.2. Effect of intra-VTA administration of OX1r antagonist SB334867 on LH stimulation-induced antinociception

To evaluate the receptor specific response of OX1 receptors in the VTA, we examined the effect of intra-VTA administration of SB334867 as a selective OX1r antagonist on LH stimulation-induced antinociception by carbachol. Two-way ANOVA for repeated measures over time followed by Bonferroni's test for %MPEs indicated a significant differences in antinociceptive responses to carbachol microinjected into the LH alone or when carbachol was concurrently administrated into the LH following different doses of intra-VTA SB334867 compared to those of respective saline-treated group [treatment effect: $F(6, 160) = 31.17$, $P < 0.0001$; time effect: $F(4, 160) = 1.368$, $P = 0.2475$; interaction: $F(24, 160) = 0.8157$, $P = 0.7130$; Fig. 2A]. However, there are no significant differences in %MPEs at any time intervals among the control and experimental groups. Moreover, as shown in Fig. 2B, one-way ANOVA followed by Newman–Keuls multiple comparison test showed that there are significant differences in mean AUC calculated values for %MPEs [$F(6, 32) = 12.39$: $P < 0.0001$] among the experimental and control (vehicles) groups. Data obtained in this experiment show that intra-VTA administration of SB334867 as an OX1r antagonist dose-dependently prevents LH stimulation-induced antinociception in rats. Intra-VTA administration of SB334867 (1, 3 and 10 but not 0.3 nmol/rat) significantly reduced %MPEs as an index for antinociception in comparison with DMSO respective control group that received carbachol (125 nmol/rat) only into the LH. On the other hand, administration of maximum dose of SB334867 (10 nmol/rat) alone into the VTA could not affect the baseline TFLs (or %MPEs) at time set intervals and/or mean AUC calculated values compared to vehicles group (Fig. 2B).

3.3. Effect of intra-accumbal administration of SB334867 on antinociception induced by carbachol microinjected into the LH

In this set of experiments, we evaluated the dose–response effects of intra-NAc administration of selective OX1r antagonist SB334867 on the antinociceptive response of carbachol microinjected into the LH during a 60-min period. Although two-way ANOVA followed by Bonferroni's test [treatment effect: $F(6,160) = 41.53$, $p < 0.0001$; time effect $F(4,160) = 1.218$, $P = 0.3053$; interaction $F(24,160) = 0.5021$, $P < 0.9750$] revealed that there are significant differences in %MPE values among the experimental and control groups in Fig. 3A, there were no significant differences in %MPEs at any time

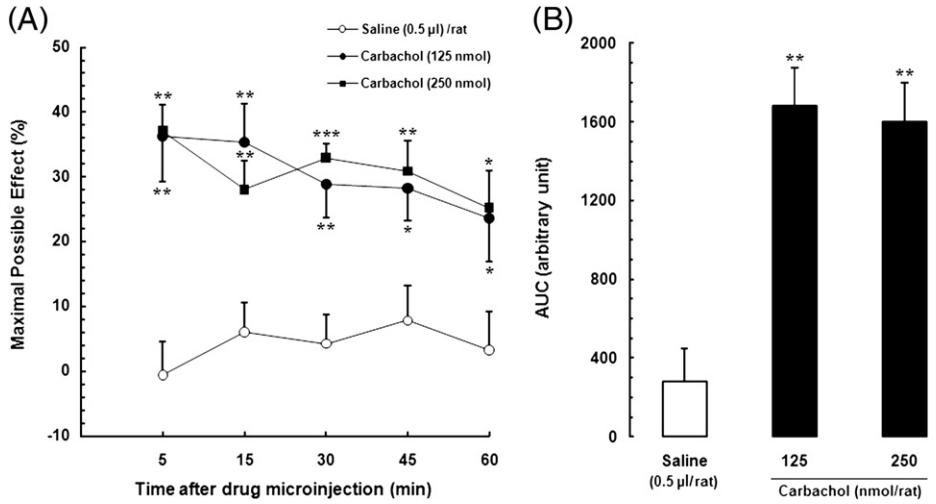

Fig. 1. Effect of unilateral administration of different doses of carbachol in the lateral hypothalamus (LH) on tail-flick latencies in the rats. (A) Maximal possible effect of two doses of carbachol at 5, 15, 30, 45 and 60 min after microinjection and (B) area under the curves (AUCs) calculated for %MPEs in 60-min period in tail-flick test as a model of acute pain shown in A. Animals received carbachol (125 and 250 nmol/0.5 µl saline) or saline as a vehicle into the LH. Each point shows the mean ± SEM for 5–6 rats. *P < 0.05, **P < 0.01, ***P < 0.001 different from the saline control group.

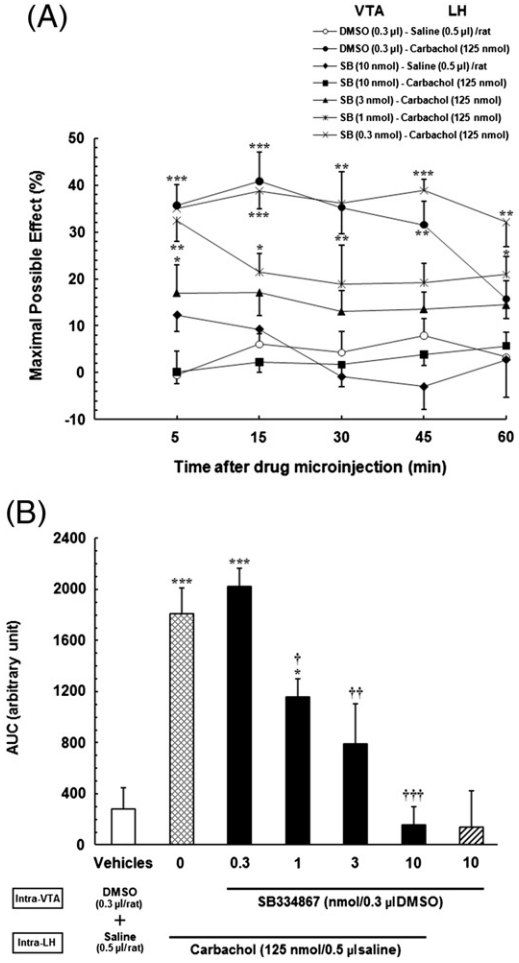

Fig. 2. Effect of unilateral microinjection of different doses of SB334867, an OX1 receptor antagonist, in the ventral tegmental area (VTA) on antinociception induced by chemical stimulation of the lateral hypothalamus (LH) as (A) maximal possible effect at 5, 15, 30, 45 and 60 min after microinjection and (B) area under the curves (AUCs) calculated for %MPEs in 60-min period in tail-flick test as a model of acute pain shown in A. Animals received SB334867 (0.3, 1, 3 and 10 nmol/0.5 µl DMSO) or DMSO as a vehicle 5 min before intra-LH administration of carbachol (125 nmol/rat). Each point shows the mean ± SEM for 6–8 rats. *P < 0.05, **P < 0.01, ***P < 0.001 different from the control (vehicle) group. † $P < 0.05$, †† $P < 0.01$, ††† $P < 0.01$ different from the respective DMSO group.

intervals. Nonetheless, one-way ANOVA followed by Newman–Keuls multiple comparison test [$F(6, 32) = 12.39$: $P < 0.0001$] showed that there are significant differences in mean AUC calculated values for %MPEs among the experimental and control (vehicles) groups. However, as shown in Fig. 3B, intra-VTA administration of SB334867 only at high doses (3 and 10 nmol/rat) could significantly reduced mean AUC (or %MPEs) in comparison with DMSO respective control group that received carbachol (125 nmol/rat) into the LH. Moreover, administration of maximum dose of SB334867 (10 nmol/rat) alone into the NAc could not affect the AUC calculated values compared to vehicles group.

On the other hand, we tried to set the AUC of the control (animals that received only 125 nmol carbachol in the LH) to 100%, and represent the remaining AUCs (animals that received different doses of SB334867 in the NAc or VTA) as % changes in their responses (Fig. 4). This figure, a log dose–response curve for carbachol in the LH and SB334867 as an OX1 receptor antagonist in either the NAc or VTA, shows that the 50% effective dose (ED50) value of SB334867 on intra-LH carbachol-induced antinociception in the VTA (1.13 nmol) is less that in the NAc (1.89 nmol). However, there was no significant difference between these two ED50 values in this study.

4. Discussion

The purpose of this study was to evaluate the involvement of orexin-1 receptors within the VTA and NAc in the antinociceptive responses induced by LH stimulation in the rat. The major findings are: (1) administration of different doses of carbachol into the LH produced antinociceptive responses in tail-flick test, (2) administration of an OX1 receptor antagonist (SB334867) into the VTA dose-dependently blocked the carbachol-induced antinociception and (3) unilateral intra-accumbal microinjection of SB334867 inhibited antinociceptive responses induced by intra-LH carbachol. Previous studies have shown that the stimulation or inactivation of LH produces antinociception (Tasker et al., 1987; Behbehani et al., 1988; Safari et al., 2009). The results in the first set of experiments are in agreement with previous studies, but we used two different doses of carbachol; although there were no significant differences in antinociceptive effects, the lower dose was the most effective dose.

Orexinergic projections originated from the LH innervate many brain regions like the VTA, NAc, amygdale and prefrontal cortex (PFC). In the VTA, orexin receptors have been settled on both dopaminergic and GABAergic neurons. Activation of orexin receptors in the

VTA mostly results in an increase in dopamine release at the level of the NAc and PFC through its projections (Sharf et al., 2010). Previous research studies have demonstrated that these projections have important roles in the reward circuitry (Taslimi et al., 2011). Some researchers have shown that the dopaminergic neurons in the VTA involve in both endogenous opioid- and morphine-induced antinociception (Altier and Stewart, 1993). Moreover, it has been shown that lesion of the VTA dopaminergic neurons affects on hyperalgesic responses and could increase self-mutilating behaviors after de-afferentation (Saade et al., 1997). Previous studies reported that stress-induced analgesia in the formalin test is mediated by SP and opioid receptors in the VTA (Altier and Stewart, 1998). Several studies have also shown that analgesia induced by intra-VTA tachykinins and intra-NAc amphetamine increases dopamine release in the NAc (Altier and Stewart, 1998). Additionally, it has been found that analgesic effect of injection of the SP analogue, DiMe-C7, into the VTA attenuates by the infusion of D1 receptor antagonist SCH23390 into the NAc (Altier and Stewart, 1998).

On the other hand, there are some reasons to believe that lesions of the substantia nigra by 6-hydroxydopamine block analgesia induced by D-amphetamine and morphine in the formalin test (Morgan and Franklin, 1990, 1991). Ghalandari-Shamami et al. (2011) showed that the NAc plays an important role in modulation of pain. This study supports the involvement of the NAc in the antinociception induced by intra-BLA administration of cannabinoid receptor agonist in tail-flick test (Ghalandari-Shamami et al., 2011). Also, it has been shown that antinociception induced by intra-VTA DiMe-C7 was decreased by intra-NAc infusions of the mixed dopamine receptor antagonist flupenthixol (Altier and Stewart, 1998). So, our findings in this study suggest that orexin-1 receptors in the VTA and NAc involve in the antinociception induced by LH stimulation, and LH orexinergic projections to these areas act, in part, through orexin-1 receptors within the VTA and NAc in pain modulation. On the other hand, it has been shown that the distribution of orexin receptors in these areas isn't the same, and the quantity of OX1 receptors in the VTA is more than that in the NAc (Sharf et al., 2010). Our results had been matched with previous findings because we observed the attenuation of the antinociceptive responses induced by LH-stimulation by blocking the OX1 receptors within the VTA, this attenuation was stronger than that in the NAc. In conclusion, it seems that the VTA orexinergic receptors are more effective on modulation of the pain descending inhibitory system than those receptors in the NAc. Additionally, we also suggest that among the brain areas which are associated with antinociceptive effects induced by LH-stimulation, VTA could be chosen as the first region involves in this phenomenon and the NAc as the second. Nevertheless, it is necessary that we extend our investigations in the future.

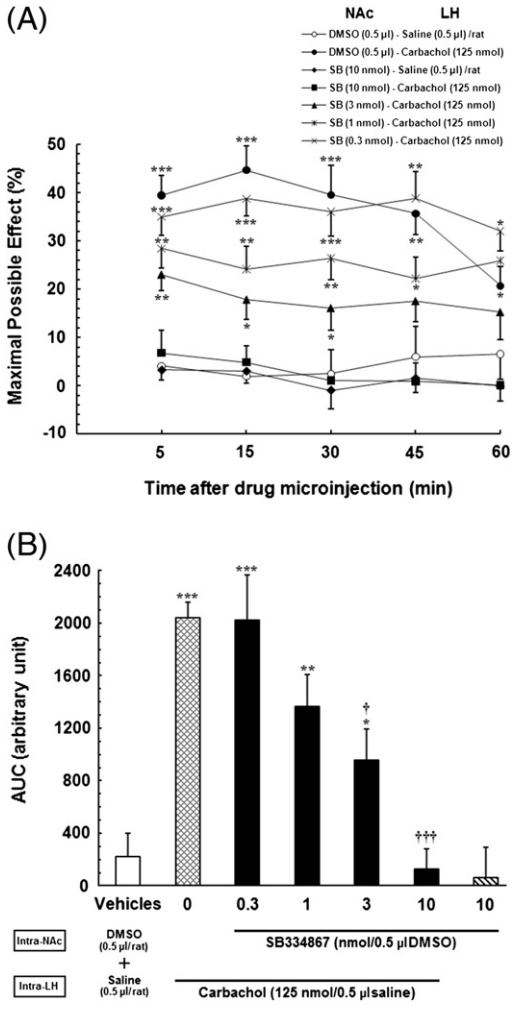

Fig. 3. Effect of unilateral microinjection of different doses of SB334867, an OX1 receptor antagonist, in the nucleus accumbens (NAc) on antinociception induced by chemical stimulation of the lateral hypothalamus (LH) as (A) maximal possible effect at 5, 15, 30, 45 and 60 min after microinjection and (B) area under the curves (AUCs) calculated for %MPEs in 60-min period in tail-flick test as a model of acute pain shown in A. Animals received SB334867 (0.3, 1, 3 and 10 nmol/0.5 µl DMSO) or DMSO as a vehicle 5 min before intra-LH administration of carbachol (125 nmol/rat). Each point shows the mean ± SEM for 6–8 rats. *P < 0.05, **P < 0.01, ***P < 0.001 different from the control (vehicle) group. † $P < 0.05$, †† $P < 0.01$ different from the respective DMSO group.

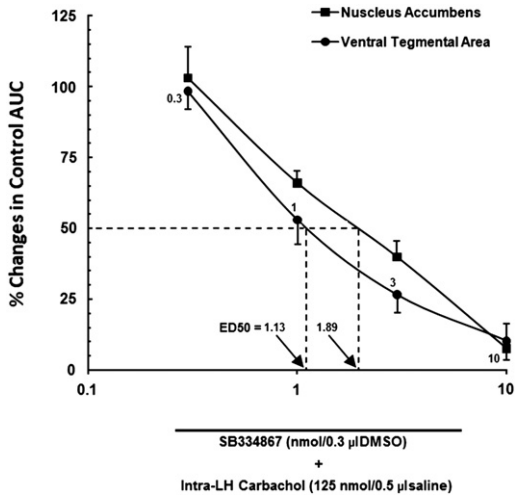

Fig. 4. A log dose–response curve for carbachol in the lateral hypothalamus (LH) and SB334867 as an OX1 receptor antagonist in either the nucleus accumbens (NAc) or ventral tegmental area (VTA). In this figure we tried to set the AUC of the control (animals that received only 125 nmol carbachol in the LH) to 100%, and represent the remaining AUCs (animals that received different doses of SB334867 in the NAc or VTA) as a % of that to generate an effective dose 50% (ED50) of SB334867 in the VTA (1.13 nmol) or NAc (1.89 nmol).

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